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Spatial and temporal expression analysis of defense-related genes in soybean cultivars with different levels of partial resistance to *Phytophthora sojae*

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Abstract

The molecular mechanisms and the defense responses associated with partial resistance to *Phytophthora sojae* in soybean are unknown. In this study, we examined correlations between the expression of defense genes with partial resistance. First, to determine whether constitutive levels of expression of defense-related genes correlated with partial resistance to *P. sojae*, northern blot analysis of seven defense-related genes in 14 cultivars with low, moderate and high levels of partial resistance was performed. Pearson's correlations between mean lesion length and mean constitutive mRNA signals for defense-related genes showed no significant association to partial resistance to *P. sojae*. These results suggested that mechanisms linked to defense-related mRNA levels expressed during infection might better explain variations in partial resistance to *P. sojae* in soybean.

Second, accumulation of four defense-related transcripts during infection was monitored in a spatial, time-course infection assay with two soybean cultivars, Conrad (high level of partial resistance) and OX 20-8 (*Rps1a*, low level of partial resistance). mRNA was isolated for Northern blot analysis from root sections harvested below, at, and above the inoculation site at 0, 6, 12, 24, 48 and 72 h after inoculation (hai) with *P. sojae*. *P. sojae* and soybean actin cDNAs were used as probes in the infected root sections to estimate relative proportions of RNA. Differential mRNA accumulation patterns for both soybean and *P. sojae* actin following *P. sojae* colonization in the three root sections of Conrad and OX 20-8 suggested that effective lesion-limiting mechanisms occurred primarily in the upper root section. Transcript levels for *PR1a,* matrix metalloproteinase (MMP) and basic peroxidase (*IPER*) at the inoculation site; and *IPER* above the inoculation site at 72 hai were significantly higher in Conrad with higher levels of partial resistance. Our results suggest that defense responses associated with accumulation of *PR1a*, *MMP, IPER* and β-1,3-endoglucanase (EGL) mRNAs may contribute to the partial resistance response to *P. sojae* in soybean.

Keywords: Partial resistance; Soybean; *Glycine max*; *Phytophthora sojae*; Defense-related genes; Pathogenesis-related genes; RNA blot analysis; Time-course infection assay; Roots; Cotyledons

1. Introduction

Phytophthora sojae Kaufmann and Gerdemann, an oomycete plant pathogen, causes root and stem rot of soybeans (*Glycine max* [L.] Merr.). Partial resistance (non-race specific) to *P. sojae* in soybean is characterized by containment of the pathogen to the lower stem and tap root [31,36]. In soybean, there is a reduction in lesion size in lines with partial resistance and this trait is quantitatively inherited [3]. Partial resistance to *P. sojae* can be evaluated in greenhouse assays by challenging cultivars or lines with a compatible pathotype and measuring the degree of root colonization [33]. Race-specific genes for resistance to *P. sojae* (*Rps* genes) have also been reported in soybean [8,31] and this resistance follows the gene-for-gene model first proposed by Flor [11].

Recent surveys on pathogenic diversity in soybean fields have shown that *P. sojae* populations in Ohio have increased

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in the number of pathotypes as well as in complexity [7,33]. This indicates that relying on the currently deployed race-specific resistance (*Rps*) genes alone for disease management presents a potential risk [7]. Thus, there is a need for more efficient incorporation of partial resistance to *P. sojae* into new or existing soybean cultivars as well as a better understanding of the molecular and/or biochemical mechanisms for this type of resistance.

Defense-related genes encode a variety of proteins including enzymes of secondary metabolism (e.g. the phenylpropanoid pathway), pathogenesis-related (PR) and regulatory proteins that control the expression of other defense-related genes [5]. These defense-related or defense-response genes are generally involved in reactions downstream from the recognition step in the signal transduction pathway [29]. Interestingly, many defense-related genes have been found to co-localize with disease resistance quantitative trait loci (QTL), indicating that those genes may account, in part, for the partial resistance phenotype. For example, defense-related genes such as phenylalanine ammonia-lyase, oxalate oxidase, chitinases and glucanases co-localize with QTLs for resistance to viral, fungal, oomycete and bacterial pathogens in different plant species [9,13,14,27,29,37]. In addition, disease resistance QTLs also map to regions carrying resistance (R) gene and resistance gene analog (RGA) clusters [2,9,13,14,29,39]. This latter observation suggests that the molecular basis of partial resistance might also be explained, in part, by the involvement of genes structurally similar to R genes that mediate receptor-mediated recognition of pathogen ligands [13,14]. However, the genetic linkage of defense-related genes and R genes with QTLs needs to be further investigated [27,29]. Recently, two QTLs for partial resistance to *P. sojae* were mapped in soybean 30–40 cM away from the closest R-gene clusters, suggesting that loci for partial resistance to *P. sojae* are different from *Rps* genes in soybean [3].

As is generally the case in many plant–pathogen systems, the biochemical and physiological mechanisms of race-specific resistance of soybean to *P. sojae* have been extensively characterized, while the defense responses associated with partial resistance are just beginning to be explored. Specifically, the roles for PR proteins in the R-gene mediated defense response are beginning to be delineated [35,40,42]. For example, ethylene has been shown to induce the activity of β-1,3-endoglucanase (glucanase), an enzyme involved in the release of glucan elicitor particles from *P. sojae* cell walls, an important event which leads to the expression of the defense response of soybeans to this pathogen [35,42]. *IPER* and matrix metalloproteinase gene (*GmMMP2*) were expressed at higher levels in soybean following inoculation with *P. sojae* [21,41]. It is not yet known whether these or other PR proteins have similar roles in the partial resistance response to *P. sojae*.

Constitutive levels of PR proteins [19,20] and PR mRNAs [38] were correlated with partial resistance in the *P. infestans*/potato and the *Alternaria solani*/tomato pathosystems, respectively. Vleeshouwers et al. [38] showed that PR-1, PR-2 and PR-5 transcripts levels were highest in non-inoculated potato cultivars with high levels of partial resistance to *P. infestans*, relative to cultivars with intermediate and low levels of partial resistance. Similarly, by comparing tomato lines varying in quantitative resistance to *A. solani*, Lawrence et al. [19,20] found that resistant lines had higher constitutive levels of chitinase and glucanase than susceptible lines. These studies indicated that higher constitutive levels of defense-related transcripts or proteins may be components of partial resistance by creating physiological conditions that limit pathogen growth [19,20,38].

In this study, we first examined constitutive levels of defense-related transcripts in non-inoculated plants of 14 soybean cultivars varying in partial resistance levels to *P. sojae*. We then performed a spatial time-course infection assay in root sections of two soybean cultivars with either high or no partial resistance to *P. sojae*. Our results suggest that: (1) there is no significant correlation between constitutive levels of defense-related gene expression and partial resistance; (2) there is a possible association of matrix metalloproteinase, *PR-1a*, basic peroxidase and glucanase transcript levels with partial resistance in infected roots; and (3) the region above the point of inoculation in the soybean root is the site for potential active *P. sojae*-lesion limiting mechanisms.

2. Materials and methods

2.1. Evaluation of partial resistance levels

Fourteen soybean cultivars for which previous greenhouse and field data on partial resistance was available were used in this study [6]. The soybean cultivars used in this study have one or more of the following *Rps* genes; *Rps*1a, *Rps*1k, *Rps*3a, and *Rps*6. Partial resistance levels of the 14 soybean cultivars were measured using the slant board assay [24]. One *P. sojae* isolate, 34.S.5.1 (vir 1a, 1b, 1k, 2, 3a, 3c, 4, 5, 6, 7), was used because it has a susceptible (compatible) reaction with all of the *Rps* genes present in the soybean cultivars used. The pathotype was determined by hypocotyl inoculation technique [7,32]. For the study, 7-day-old soybean seedlings were inoculated 2 cm below the beginning of the root zone [24]. The lesion length (mm) from the inoculation point up the hypocotyl was measured 5–7 days following inoculation [24]. For each experiment, 10 seedlings of each cultivar were inoculated for each replication. There were three replications within each experiment and this was completed three times.

2.2. Constitutive and spatial time-course infection levels of defense related transcripts

Constitutive levels of defense related transcripts were measured on non-inoculated roots and cotyledons of the 14 soybean cultivars. Plants were grown as described above and roots and cotyledons of 7-day-old plants were collected from the pots, washed to remove vermiculite and stored at −80 °C. RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the supplier's instructions. This experiment was repeated once.

For the time-course infection assay, the levels of plant defense-related transcripts were measured in root sections of two soybean cultivars, Conrad and OX 20-8. Roots were inoculated with *P. sojae* isolate 34.S.5.1 in a slant board assay (described above) and root samples were collected at 0, 6, 12, 24, 48 and 72 h after inoculation (hai) and from a mock inoculated control 6 hai. A 1.5 cm root section was taken surrounding the inoculation site as well as 1.5 cm from immediately below and above at each time point beginning at 6 hai. Corresponding root sections were pooled from 20 plants, frozen in liquid nitrogen and stored at −80 °C until RNA was extracted as described above. This experiment was repeated once.

2.3. RNA blot analysis

RNA concentrations were determined using PicoGreen (Molecular Probes, Eugene, OR, USA). RNA samples (10 μg) were mixed with the Northermax glyoxal gel loading solution (Ambion, Austin, TX), loaded on a 1.4% agarose gel and separated by electrophoresis under denaturing conditions [30]. RNA was transferred to Immobilon-NY nylon membranes (Millipore, Bedford, MA) following the manufacturer's protocol, and fixed using a u.v. transilluminator. Pre-hybridization and hybridization steps were performed at 65 °C using modified Church buffer (0.36 M Na_2HPO_4, 0.14 M NaH_2PO_4, 1 mM EDTA, 7% SDS). Radiolabelled probe DNA was synthesized according to Feinberg and Volgenstein [10]. Blots were hybridized overnight at 65 °C, then washed to a stringency 0.5× SSC/0.5% SDS, at 65 °C. Hybridization was detected and quantified using a STORM 840 phosphorimager and ImageQuaNT software (Molecular Dynamics, Sunnyvale, CA). Ribosomal RNA band intensities from the ethidium bromide-stained gels prior to transfer were quantified using the ImageJ 1.29x software (Wayne Rasband, National Institutes of Health, USA).

2.4. cDNA probes

Primers to amplify portions of the coding sequences for the soybean matrix metalloproteinase (MMP), chalcone synthase (CHS), Class I chitinase (BCHIT), β-1,3-endoglucanase (EGL), a basic peroxidase (IPER), phenylalanine ammonia-lyase (PAL), *PR1a* and actin (ACT) were designed based on sequences available from GenBank, using MacVector ver 6.5 (Oxford Molecular Ltd, Madison, WI) (Table 1). RNA from 8- and 9-day-old soybean roots and hypocotyls of the *P. sojae* inoculated cultivar Williams79 (*Rps*1c), was used to amplify target sequences using the RT-PCR Retroscript kit (Ambion, Austin, TX) following the manufacturer's instructions. The cDNAs were cloned into either pCR 2.1-TOPO (Invitrogen, Carlsbad, CA) or pGEM-T Easy (Promega, Madison, WI) according to the supplier's instructions. Plasmid insert sequences were determined (Plant–Microbe Genomics Facility at the Ohio State University, Columbus, OH) and aligned with the GenBank sequences using ClustalW (ver. 1.81) to verify the identity of insert DNA. The relative proportion of pathogen RNA in each root section was estimated with *P. sojae* actin cDNA, obtained from M. Gijzen, Agriculture and Agri-Food Canada, London, Ontario [28].

Table 1
Soybean defense related genes, GenBank accession numbers and primers for coding sequences used to design cDNA probes for Northern blot analysis

Gene	GenBank accession No.	Primers used in this study	Probe size (bp)	Reference
Chalcone synthase (CHS)	X53958	5′-TCAACCCAAGTCCAAGATTACCC-3′ 5′-CCAAGGTGTCCATCAATAGCCC-3′	420	[1]
Class I Chitinase (BCHIT)	AF202731	5′-GTGGAGCGTTATGCCCAAATAG-3′ 5′-CTTGAATGACACGGTTGCGTC-3′	596	[15]
β-1,3-Endoglucanase (EGL)	M37753	5′-CAATCAAGCCAACATTCGCAG-3′ 5′-GCAGCATAAACAGCATCAACCG-3′	600	[35]
Basic peroxidase (IPER)	AF007211	5′-CTCTCAGGTGCTCATACATTCGG-3′ 5′-TACACAACAAGTAGTGGCGGGC-3′	598	[41]
Phenylalanine ammonia-lyase (PAL)	X52953	5′-GCTAAGAAGTTGCATGAGATTG-3′ 5′-GGACGAGTTCACATCCTTC-3′	746	[12]
PR1-a	AF136636	5′-TTGTGTGATGTGTGTGTTGGGG-3′ 5′-AGTGATGAAAGTGCCTCCGTTATC-3′	583	[17]
Matrix metalloproteinase (MMP)	AY057902	5′-CCCACACAAACCTCACATACGG-3′ 5′-AAACTCACTCCCAAACGCCC-3′	529	[21]
Actin (ACT)	AF049106	5′-TGCCATCCTCCGTCTTGACTTAGC-3′ 5′-GCCTCATCATACTCAGCCTTTGC-3′	549	[16]

2.5. Statistical analyses and standardization of hybridization signals

An analysis of variance (ANOVA) for lesion length was done using the GLM procedure in SAS (Cary, NC). Differences among cultivars were separated using Fishers Protected least significant difference (LSD, $P<0.05$). Signals for constitutive mRNA levels for each gene were standardized to rRNA levels to correct for differences in RNA loading and to facilitate comparisons among blots. Pearson's correlations between the relative mRNA levels for individual defense related genes and lesion length were then calculated.

Gene expression data from the spatial time-course infection assay was also corrected for the increasing level of *P. sojae* RNA in the blot, because later time points had a higher percentage of *P. sojae* mRNA than soybean RNA. Here, hybridization signals of the defense-related transcripts were divided by the corresponding signal for soybean actin mRNA to obtain corrected values. Standardized values were used in the analysis of variance (ANOVA) to test for differences in defense gene expression over time and space using GLM procedure.

3. Results

3.1. Variation in levels of partial resistance in soybean cultivars

Lesion lengths among the 14 cultivars following inoculation with *P. sojae* isolate 34.S.1.1 were significantly different ($P<0.001$, Table 2). At least three groups could be determined using Fishers Protected LSD of the mean lesion length: Soybean cultivars with low levels of partial resistance (largest lesion lengths) were OX 20-8 and Harosoy, while Defiance and Croton 3.9 had moderate levels of partial resistance, and Darby and Athow had the highest (smallest lesion lengths) levels of partial resistance (Table 2).

Table 2
Partial resistance levels[a] to *Phytophthora sojae* (34.S.5.1) in 14 soybean cultivars

Cultivars (*Rps*)	Lesion length (mm)
OX 20-8 (*Rps*1a)	54.6
Harosoy (*Rps*7)	44.9
Sloan	43.8
Williams	42.3
OHFG1 (*Rps*3a)	41.6
Defiance (*Rps*3a)	41.3
Croton 3.9	38.1
Stressland	36.6
Resnik (*Rps*1k)	35.8
Conrad	30.5
Conrad 94 (*Rps*1k, *Rps*6)	29.5
General (*Rps*1k)	22.5
Darby (*Rps*1k)	21.9
Athow (*Rps*1k)	18.5
Mean	36.3
LSD ($P<0.05$)[b]	6.2

[a] Partial resistance levels were measured from root lesions on 10 plants per replication taken 7 days after inoculation with *P. sojae*. Each experiment was comprised of three replications and the experiment was repeated once.
[b] LSD indicates Fisher's protected least significant difference.

3.2. Analyses of constitutive levels of defense-related transcripts

Relative transcript abundance of defense-related mRNAs did not differ significantly in roots or cotyledons of the 14 soybean cultivars with different levels of partial resistance to *P. sojae* (Fig. 1). Transcripts for each of the defense-related genes could be detected in non-inoculated roots of all cultivars by Northern blot analysis (Fig. 1). In cotyledons, *PR1a* mRNA could only be detected in Sloan, OHGF1 and Defiance, and the glucanase transcript was significantly less abundant in cultivars OHFG1 and Harosoy. The high levels of some transcripts that appeared in some cultivars as seen in Fig. 1 (e.g. the *PR1a* transcript in Athow roots) were not reproducible in the second experiment. However, visibly increased transcript levels for *BCHIT* in OHFG1 (moderate levels of partial resistance) and Harosoy (moderately susceptible) cotyledons were seen in both experiments. Pearson's correlations between relative transcript levels and lesion lengths did not indicate any significant correlation between expression of any of the defense-related genes and lesion length in either roots or cotyledons (Table 3).

3.3. Spatial time-course infection assay of P. sojae in soybean roots

Because partial resistance to *P. sojae* was reflected by reduced colonization following pathogen inoculation, the response of a cultivar with a relatively high level of partial resistance (Conrad) was compared with that of a fully susceptible cultivar (OX 20-8). Root tissue at and below the inoculation site in both Conrad and OX 20-8 appeared yellow-brown and soft by 48 hai. At this time, root tissue above the inoculation point was yellow, soft and water-soaked in OX 20-8, while no symptoms were visible in Conrad. Hand dissection of roots at 48 hai indicated that the internal lesion in OX 20-8 was twice as long as in Conrad (data not shown).

P. sojae actin mRNA was detected in inoculated roots as early as 24 hai at the site of inoculation and at 48 hai above and below the inoculation site (Fig. 2). *P. sojae* actin levels were significantly higher ($P=0.04$) in OX 20-8 than Conrad above the point of inoculation (Fig. 2), but levels were similar in the two cultivars for below and at the inoculation site. These data suggest that the sections below and at the site of inoculation have less soybean biomass compared to the section above the inoculation site.

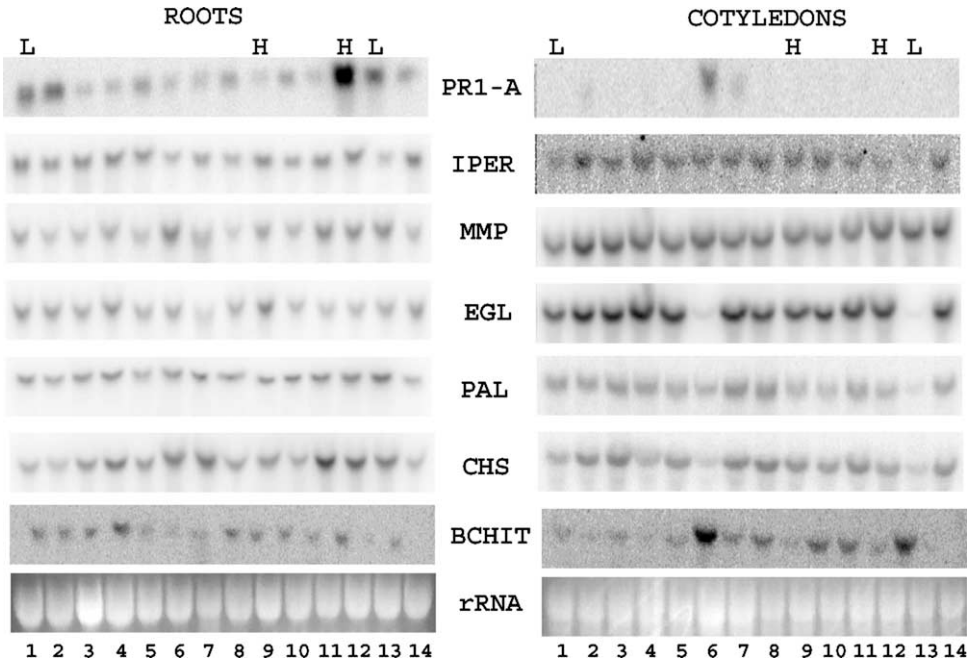

Fig. 1. Northern blots of defense-related gene expression in non-inoculated roots and cotyledons of soybean cultivars varying in levels of partial resistance to *P. sojae*. Letters 'L' and 'H' at the top of the figure show the positions of the two cultivars with lowest and highest levels of partial resistance, respectively. IPER: basic peroxidase; MMP: matrix metalloproteinase; EGL: glucanase; PAL: phenylalanine ammonia-lyase; CHS: chalcone synthase; BCHIT; class I chitinase. Ethidium bromide-stained 28S rRNA bands are shown as RNA loading controls. Similar amounts of rRNA were present on all gels. Soybean cultivars were loaded on the gels as follows: OX20-8 (1), Sloan (2), Resnik (3), Darby (4), Croton3.9 (5), OHFG1 (6), Defiance (7), Conrad94 (8), General (9), Conrad (10), Stressland (11), Athow (12), Harosoy (13), and Williams (14).

3.4. Defense-related mRNA accumulation in response to P. sojae in soybean roots

Transcript accumulation for *PR1a*, *EGL*, *IPER* and *MMP* were determined in a spatial, time-course infection assay in soybean roots of cultivars Conrad and OX 20-8 inoculated with *P. sojae* (Fig. 3). The expression of all four transcripts varied with time after inoculation and among the different root sections indicating that *P. sojae* infection induced changes in gene expression over time and space in both cultivars. For each transcript, ANOVA indicated that the observed differences in mRNA accumulation were highly significant among time points in each of the root sections ($P<0.002$) with one exception. Differences in transcript accumulation over time for *IPER*, below the site of inoculation, were not significant ($P=0.064$). No significant differences in transcript accumulation between wounded and non-inoculated controls were found, indicating that wounding did not induce gene expression of these defense related genes in soybean roots at 6 hai (data not shown).

In addition to spatial and time-course differences in mRNA accumulation found for defense-related genes, differences in accumulation occurred between the two cultivars (Fig. 3). Above the inoculation site (Fig. 3, panels 3), transcript levels were higher in Conrad at 72 hai for *IPER* for both

Table 3
Pearson's correlation coefficients to test for association between lesion length and constitutive transcript levels for defense-related genes assayed in roots and cotyledons

Transcript	Roots	Cotyledons
PR1-a	−0.079	0.297
Basic peroxidase	−0.126	0.072
Matrix metalloproteinase	0.074	−0.304
β-1,3-Endoglucanase	−0.094	−0.293
Phenylalanine ammonia-lyase	−0.017	0.508
Chalcone synthase	−0.193	0.035
Class I chitinase	−0.139	0.086

No significant differences were found at $P<0.05$ or $P<0.01$ levels.

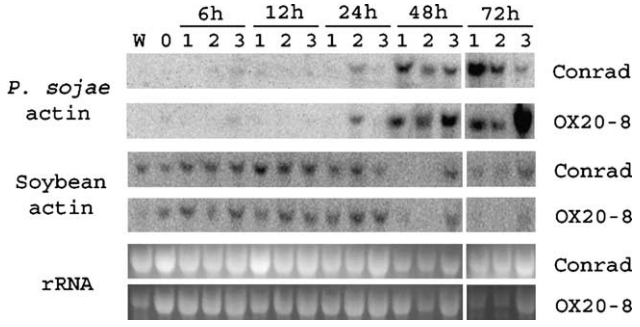

Fig. 2. Northern blot of a time-course assay of both *P. sojae* and soybean actin mRNA accumulation in inoculated root sections of soybean cultivars Conrad and OX 20-8. Expression profiles of tissue collected from: (1) The lower root section (below the inoculation point), (2) the site of inoculation, and (3) the upper root section (above the inoculation point).

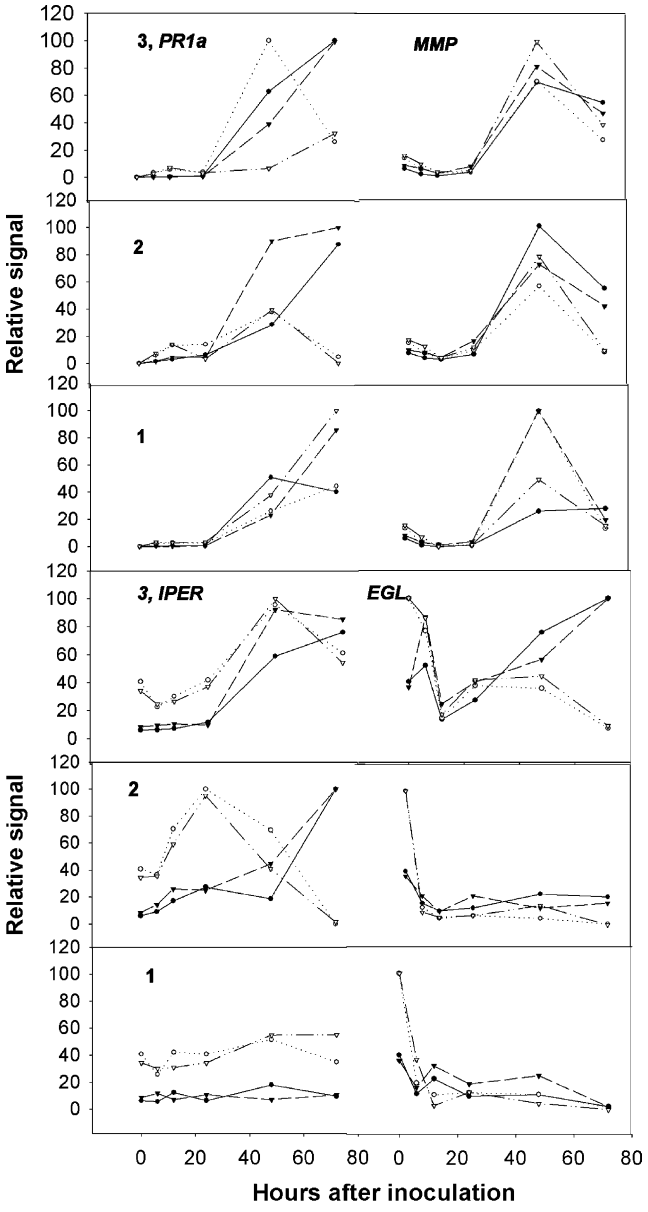

Fig. 3. Time-course of *PR1a*, matrix metalloproteinase (MMP), basic peroxidase (*IPER*), and β-1,3-endoglucanase (EGL) mRNA accumulation in root sections from two independent experiments of soybean cultivars Conrad (closed dark circles and triangles) and Ox 20-8 (open circles and triangles) inoculated with *P. sojae*. Expression profiles using tissue collected from the (1) lower root section (below the inoculation point), (2) site of inoculation, and (3) upper root section (above the inoculation point).

replicates. At the inoculation site (Fig. 3, panels 2), transcript accumulation for *PR1a, MMP,* and *IPER* were also higher in Conrad than in Ox 20-8 at 72 hai. Transcript levels were not different between the two cultivars for *EGL*, *MMP* and *PR1a* below the site of inoculation, for *EGL* at the site of inoculation or for *EGL*, *MMP* and *PR1a* above the site of inoculation (Fig. 3). These data suggested a differential response of the partially resistant Conrad to *P. sojae* manifested 48–72 hai.

4. Discussion

Differential constitutive expression of defense-related genes could not be correlated with levels of partial resistance to *P. sojae* in soybean, in contrast to previous results with other plant pathogen systems [19,20,38]. Although the 14 soybean cultivars used in this study were separated into low, moderate and high levels of partial resistance, no correlation between constitutive transcript levels for the seven defense-related genes and partial resistance to *P. sojae* was found in either roots or cotyledons. These results suggested that the mechanisms associated with partial resistance may be linked to the presence of the pathogen. This is in contrast to previous studies in potato and tomato in which clearly higher constitutive levels of some defense-related mRNAs correlated with partial resistance to *P. infestans* and *A. solani*, respectively [19,20,38].

Spatial analysis of *P. sojae* growth using a pathogen-specific probe indicated that the zone above the point of inoculation of the soybean root is the site for *P. sojae*-lesion limiting mechanisms in the soybean cultivar, Conrad. The nature of these rate-limiting mechanisms was investigated using a time-course northern blot analysis for several defense related genes. Temporal and spatial changes in mRNA accumulation for all the defense-related genes evaluated occurred in both cultivars following inoculation with *P. sojae*. However, with the experimental and statistical approaches used in this study, we would not identify small differences in transcript levels associated with partial resistance.

EGL is an enzyme involved in the release of glucan elicitor particles from *P. sojae* cell walls, which is an important event in the defense response of soybeans to this pathogen [35,42]. Glyceollin accumulation is an important component this defense response [35,42]. In this study, *EGL* mRNA levels in the upper root section at 72 hai were significantly higher in the partially resistant cultivar. The involvement of the glyceollin response in partial resistance of soybean to *P. sojae* is still not clear. In an earlier study, glyceollin accumulation in *P. sojae*-inoculated roots and hypocotyls of soybean cultivars and lines with different levels of partial resistance did not account for expression of this type of resistance [25]. However, later studies demonstrated that the restriction of *P. sojae* lesions correlated with increase glyceollin concentration [18,22,42], suggesting that enhanced resistance could be obtained by modulation of glyceollin levels. Studies which examine the expression of additional genes associated with glyceollin production in soybeans with varying levels of partial resistance will aid in delineating the role of this phytoalexin in the partial resistance defense response.

Plant peroxidases are involved in pathogen defense responses, among other physiological processes [4]. The *IPER* cDNA, which encodes a putative peroxidase, was originally isolated from a *P. sojae*-infected soybean

hypocotyl cDNA library [41]. *IPER* was shown to be expressed predominantly in the R-gene mediated resistance response to *P. sojae*, but also increased during the susceptible response [41]. Recently, Park et al. [26] showed that distal protection against *P. sojae* colonization of cotyledon tissues treated with a glucan elicitor prior to infection correlated with the accumulation of a peroxidase isozyme. The results from this study indicate that *IPER* may play a role in partial resistance of soybean to *P. sojae* since expression levels were higher in Conrad than the susceptible cultivar both above and at the point of inoculation.

PR-1 has been shown to inhibit both the germination of *P. infestans* zoospores in vitro and lesion growth in vivo in tomato [23]. PR-1 expression may have some effect against *P. sojae* in soybean as well, as significant differences between Conrad and OX 20-8 in *PR1a* transcript levels were found at the inoculation site. However, based on *P. sojae* actin levels, there was more pathogen RNA in OX 20-8 than in Conrad above the inoculation point only. This suggests that the higher levels of *PR1a* mRNA at the inoculation site may contribute to less pathogen biomass above the site of inoculation in Conrad.

Recently, Smart et al. [34] reported that partial resistance to *P. infestans* in tomato is independent of the salicylic acid, ethylene and jasmonic acid signaling pathways and suggested that partial resistance to *P. infestans* may be due to as yet uncharacterized defense mechanisms. In another study, salicylic acid and jasmonic acid had no effect on the accumulation of *MMP* transcripts [21]. The four enzymes that we examined in this study do not fully explain this defense response; thus, it seems quite possible that partial resistance to some pathogens, such as *P. sojae* or *P. infestans*, could be relatively complex and different from those associated with R-gene mediated resistance. Microarray gene expression profiling studies are underway to examine the global gene expression profile of both soybean cultivars varying in partial resistance levels, and *P. sojae*, and should help in delineating possible defense responses. Examining the defense response in a one gene at a time scenario is both costly and tedious. Information gained from these studies is nonetheless important for designing experiments that use high throughput technologies to examine expression of multiple gene families. For example, this study indicates the most important portion of the root to focus on for expression of partial resistance is above the inoculation point and measurable differences can be detected several days after inoculation with the pathogen.

In summary, we have shown that, in non-inoculated roots and cotyledons, constitutive mRNA levels for defense-related genes do not correlate with partial resistance levels to *P. sojae* in soybean. Furthermore, following inoculation of soybean roots with *P. sojae*, mRNA levels for *PR1a, MMP*, and *IPER* at the inoculation site, and *IPER* above the inoculation point, were higher in the cultivar with greater levels of partial resistance to *P. sojae*, suggesting that mechanisms associated with expression of those genes during infection may contribute to this response in this pathosystem. These results, showing the importance of the upper root of the soybean as the tissue responsible for lesion-limiting and *P. sojae*-growth restricting mechanisms, will allow us to focus future work to define the partial defense response to the root region at and above the inoculation point. Identifying the genes responsible for expression of partial resistance of soybean to *P. sojae* remains a challenge. Further and more detailed characterization of how partial resistance to *P. sojae* is controlled in soybean, at the molecular level, will advance our understanding of quantitative disease resistance in plants and should provide additional tools to incorporate and deploy this type of resistance in commercially viable soybean cultivars.

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